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Process for selective oxidation of alc hols and Novel carbohydrate aldehydes

[1] The invention relates to the production of aldehydes by selective oxidation of alcohols. The oxidation is carried out using an oxidising agent in the presence of a catalytic amount of a di-tertiary-alkyl nitroxyl compound, especially 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO).

[2] Such a process in which TEMPO is reoxidised by chemical means is known from a review by De Nooy in *Synthesis* 1996, 1153-1174.

[3] It was found according to the invention that the oxidation of alcohol functions, especially primary alcohol functions, can be carried out without using chlorine-based oxidising agents and with the use hydrogen peroxide or oxygen as the ultimate oxidising agent. The oxidation according to the invention is performed using enzymes and/or metal complexes. This oxidation, when carried out on primary alcohols, surprisingly results in formation of aldehydes, without further oxidation to carboxylic groups under appropriate conditions. The aldehydes may be present in the (hemi)acetal form and related structures. An adaptation of the oxidation of the invention can be used to oxidise secondary alcohols, especially carbohydrates, to keto derivatives. The process of the invention is further defined by the characterising features of the appending claims.

[4] In the following description, reference is made to TEMPO only for the sake of simplicity, but it should be understood that other di-tert-alkyl nitroxyls, such as 2,2,5,5--tetramethylpyrrolidine-N-oxyl (PROXYL) and 4-hydroxy-TEMPO and derivatives thereof and those described in WO 95/07303 can be substituted for TEMPO. These di-tert-alkyl nitroxyls are especially suitable for selectively oxidising primary alcohols to aldehyde functions, in particular in the presence of secondary alcohol functions that should not be oxidised. Less sterically hindered nitroxyls, such as 4,4-dimethyl-oxazolidine-N-oxyl (DOXYL), are suitable for preferentially oxidising secondary alcohols to keto functions, for example in the production of keto cellulose or keto starch.

[5] A catalytic amount of nitroxyl is preferably 0.1-2.5% by weight, based on the primary alcohol, or 0.1-2.5 mol% with respect to the primary alcohol. The nitroxyl may also be immobilised, e.g. by coupling of the hydroxyl group of 4-hydroxy-TEMPO to a suitable carrier, or in the form of a polymeric nitroxyl such as:

$-[(CH_3)_2C-NO.-C(CH_3)_2-A-]_n-$, wherein A may be an alkylene group and/or a hetero-atom, and n is a number form e.g. 10 up to several hundreds.

[6] The process of the invention results in oxidation of primary alcohols initially to the corresponding aldehydes. If required the primary products can be further oxidised to the corresponding carboxylic acids by using known oxidising agents such as hypochlorite, chlorite, hydrogen peroxide or by using TEMPO-mediated oxidation under more vigorous conditions such as an increased temperature e.g. from 40-80 °C, or for prolonged exposure to the reaction conditions.

[7] The present process is especially favourable for the selective oxidation of primary hydroxyl groups in alcohols having a secondary alcohol function in addition to the primary alcohol, such as 1,6-octanediol, 1,9-octadecanediol, steroid hormones, sugar alcohols, glycosides (flavour precursors), and in particular carbohydrates having primary alcohol functions. The carbohydrates may be monosaccharides, such as glucose, fructose, disaccharides, such as sucrose, maltose, lactose, oligosaccharides and polysaccharides. The oligo- and polysaccharides may be of any type, e.g. glucans such as starch, starch components (i.e. amylose, amylopectine, dextrins), cellulose, chitin, lichenin etc., furanofructans such as inulin and levan, galactans, arabinogalactans, furanoid pentosans (xylans), (galacto)mannans (guar, locust bean gum), bacterial exopolysaccharides (EPS) and the like and derivatives of such carbohydrates, such as hydrolysates. These oligo- and polysaccharides include heterosaccharides, i.e. those which have different structural units, even if those different units themselves may not have primary hydroxyl groups such as uronic acid units, e.g. in xanthan and carbohydrates derived form algae.

[8] A distinct group of compounds suitable for oxidation with the present process consists of hydroxyalkylated carbohydrates such as hydroxypropyl cellulose, hydroxyethyl starch or hydroxyethyl inulin, which result in an alternative way for producing formylalkyl carbohydrates. Other suitable carbohydrate substrates in which at least a part of the (6-) hydroxymethyl groups are intact, include for example (2- and 3-) carboxymethyl carbohydrates.

[9] The oxidation of carbohydrates containing primary hydroxyl groups results in the corresponding carbohydrates containing aldehydes and, if desired, to carboxylic acids, with intact ring systems. Examples include α-1,4-glucan-6-aldehydes, β-1,4-glucan--6-aldehydes, β-2,1-fructan-6-aldehydes and β-2,6-fructan-1-aldehydes. These

products are useful intermediates for functional carbohydrates wherein the aldehyde groups are further reacted with e.g. amine compounds and the like. They are also useful intermediates for crosslinked carbohydrates, in which the aldehyde groups are further reacted with e.g. diamine reagents.

[10] The catalysts to be used according to the invention are oxidoreductases or other enzymes that are capable of oxidation in the presence of a suitable redox system. Oxido-reductases, i.e. enzymes capable of oxidation without the presence of further redox systems, to be used in the process of the invention include peroxidases and oxidases, in particular polyphenol oxidases and laccase. Certain hydrolases, such as phytase, can be used when a further redox system is present such as a metal complex, e.g. vanadate. Metal complexes as such, without an enzyme protein, can also be used; examples include copper and iron complexes with porphyrins, phenanthrolins, polyamines such as EDTA, EGTA and the like. The metal-assisted enzymes and metal complexes require hydrogen peroxide, alkyl and ar(alk)yl hydroperoxides (such as tert-butyl hydroperoxide) or chlorite as an ultimate electron acceptor.

[11] Peroxidases (EC 1.11.1.1 – 1.11.1.11) that can be used according to the invention include the peroxidases which are cofactor-independent, in particular the classical peroxidases (EC 1.11.1.7). Peroxidases can be derived from any source, including plants, bacteria, filamentous and other fungi and yeasts. Examples are horse-radish peroxidase, soy-hull peroxidase, myelo peroxidase, lactoperoxidase, *Arthromyces* and *Coprinus* peroxidases. Several peroxidases are commercially available. The per-oxidases require hydrogen peroxide as an electron acceptor.

[12] Polyphenol oxidases (EC 1.10.3.1) include tyrosinases and catechol oxidases, such as lignine peroxidase. Suitable polyphenol oxidases may be obtained from fungi, plants or animals. The polyphenol oxidases require oxygen as an electron acceptor. Laccases (EC 1.10.3.2) are sometimes grouped under the polyphenol oxidases, but they can also be classified as a distinct group, sometimes referred to as p-diphenol oxidases. Laccases can be derived from plant sources or from microbial, especially fungal, sources. The laccases also require oxygen as an electron acceptor.

[13] The process of the invention can be performed under relatively mild conditions, e.g. at a pH between 2 and 10, and at a temperature between 15 and 60°C (both depending on the particular enzyme or metal complex). The reaction medium can be an

aqueous medium, or a homogeneous mixed medium, e.g. of an alcohol/water or an ether/water mixture, or a heterogeneous medium, e.g. a mixture of water and a water-immiscible organic solvent such as a hydrophobic ether, a hydrocarbon or a halogenated hydrocarbon. In the latter case, the enzyme and/or the nitroxyl and the oxidising agent may be present in the aqueous phase and the alcohol substrate and the aldehyde or ketone product may be present in the organic phase. If necessary, a phase transfer catalyst may be used. This type of reaction is suitable e.g. for the oxidation of steroids. The reaction medium can also be a solid/liquid mixture, in particular when the enzyme of the nitroxyl are immobilised on a solid carrier. A heterogeneous reaction medium may advantageous when the substrate or the product is relatively sensitive or when separation of the product from the other reagents may present difficulties.

[14] The invention also pertains to novel carbohydrate oxidation products and derivatives thereof, which can be obtained with the process of the invention. These include polysaccharides in which at least 1 hydroxymethyl per 100, especially per 50 or even per 25, monosaccharide units has been converted to a carbaldehyde group, whether or not in hemiacetal or similar form, with the proviso that on average each molecule contains at least 1 carbaldehyde group other than a possible (hemiacetalised) aldehyde group at the reducing end of an oligo- or polysaccharide. The carbaldehyde group is preferably present in chain (backbone) units, rather than in branch units. Not included in this at least carbaldehyde group per 100 (50, 25) units are carbaldehyde groups derived from terminal galactose units, which are obtainable by oxidation with galactose oxidase. The novel products include glycoside derivatives, i.e. products which, in addition to an acetalised end group have at least one carbaldehyde group obtainable by oxidation of non-galactose hydroxymethylene groups. In the products of the invention, the monosaccharide rings that carry the carbaldehyde group are largely intact, and the number of aldehyde groups is greater, especially more than two times greater, than the number of carboxyl groups (other than introduced carboxyalkyl groups). Such products are not accessible by prior art oxidation methods, which invariably lead to at least partial further oxidation to carboxyl groups. The only common carbohydrate derivatives having a predominant content of aldehyde groups are periodate-type oxidation products of starch, cellulose and the like, in which the rings bearing the aldehyde groups are broken. The aldehyde carbohydrates covered by the present invention are of any type other than

the cellulose or pentosan type (or derivatives such as carboxymethylated, alkylated, hydroxyalkylated cellulose).

[15] The novel derivatives of the invention are very suitable as thickeners, viscosifiers, stabilisers for emulsions and the like, and especially as starting materials for further functionalisation, especially with alcohols, amines, and other agents capable of coupling with an aldehyde function. Such agents include crosslinking agents (diamines, diols and the like), which can be used to crosslink the carbohydrates or to couple them to amino acids, proteins, active groups etc.

[16] The invention also pertains to derivatives obtained by coupling of the aldehyde carbohydrates described above with e.g. amines as defined in the appending claims.

[17] **Example**: *Production of 6-aldehyde starch*

Two grams of starch were gelatinised in 100 ml of water at 100°C. The solution obtained was cooled to 22°C. To this solution were added 25 mg TEMPO (0.13 mmol) and 40 mg of peroxidase (HRPO). The pH was adjusted to 5 with acetic acid (0.1 M). A hydrogen peroxide solution (1.5 ml 30% in 50 ml) was added dropwise (2 ml per h). No pH adjustment was necessary. After 25 h a sample was analysed by addition of hydroxylammonium chloride (back-titration of released acid). According to this indirect analysis, 30% of C6-aldehyde starch was formed, which was confirmed by ^{13}C NMR (intensity of C6 signal with respect to C1 of anhydroglucose unit).

[18] **Example**: *Production of 6-aldehyde cellulose*

One gram of totally bleached sulphate pulp fibers (SCA Östrand mill, dry weight) was suspended in 100 ml of water. To this suspension were added 18 mg of TEMPO (0.1 mmol) and 9 mg of peroxidase (HRPO), type VI (290 units/mg). The pH was adjusted to 5.1 with aqueous acetic acid (0.1 M). A hydrogen peroxide solution (1.5 ml 30% in 50 ml) was added stepwise (30-50 µl every 2 minutes) for 8 hours. After peroxide addition the pH decreased, but it returned to its original value (5.5) after a few moments; therefore, no pH adjustment was necessary during the reaction. After 21 h a sample was analysed by addition of hydroxylammonium chloride. The pH of the mixture was brought to pH 11.6 by addition of aqueous NaOH, and back-titrated with 0.1 M HCl. According to this analysis, the sample contained 26% of C6-aldehyde cellulose.

Claims

1. A process for producing aldehydes or ketones by oxidising a primary or secondary alcohol, respectively, using an oxidising agent and a catalytic amount of a nitroxyl compound, *characterised* in that the alcohol is oxidised in the presence of a metal complex and/or an enzyme capable of oxidation.

2. A process according to Claim 1, wherein a primary alcohol is oxidised using a di–tert–nitroxyl compound, especially 2,2,6,6–tetramethylpiperidin–1–oxyl (TEMPO).

3. A process according to Claim 1 or 2, wherein the enzyme capable of oxidation is an oxidoreductase.

4. A process according to Claim 3, wherein the enzyme is a peroxidase, especially horse radish, soy–bean, lignin peroxidase or myelo– or lacto–peroxidase, and the oxidising agent is hydrogen peroxide.

5. A process according to Claim 3, wherein the enzyme is a polyphenol oxidase or a laccase and the oxidising agent is oxygen.

6. A process according to Claim 1 or 2, wherein the enzyme is a hydrolase, especially phytase, in the presence of a metal compounds, such as a vanadate.

7. A process according to Claim 1 or 2, wherein the metal complex is a copper or iron complex with a nitrogen compound such as a porphyrin, phenanthrolin or EDTA.

8. A process according to any one of Claims 1–7, wherein the primary alcohol is a carbohydrate, especially cellulose, hemicellulose, starch, inulin or a derivative thereof.

9. A process according to any one of Claims 1–7, wherein the primary alcohol is a hydroxyalkylated carbohydrate, or a carbohydrate glycoside.

10. A process according to any one of Claims 1–7, wherein the primary alcohol is steroid compound.

11. An oxidised carbohydrate, the carbohydrate being selected from disaccharides, oligosaccharides and polysaccharides of the α–glucan, mannan, galactan, fructan, and chitin types and carbohydrate glycosides, containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 50 monosaccharide units and per average molecule, or a chemical derivative thereof.

12. An oxidised carbohydrate according to Claim 11, containing at least 5 monosaccharide units per average molecule.

13. An oxidised carbohydrate according to Claim 11 or 12, which contains 1 to 50 cyclic monosaccharide chain group carrying a carbaldehyde group per 50 monosaccharide units and per average molecule.

14. An oxidised carbohydrate according to any one of Claims 11–13, which contains α–1,4–6–oxo–anhydroglucose units.

15. A carbohydrate derivative according to any one of Claims 11–14, in which derivative at least a part of the carbaldehyde groups has been converted to a group with the formula –CH=N–R or $-CH_2-NHR$, wherein R is hydrogen, hydroxyl, amino, or a group R^1, OR^1 or NHR^1, in which R^1 is C_1-C_{20} alkyl, C_1-C_{20} acyl, a carbohydrate residue, or group coupled with or capable of coupling with a carbohydrate residue.

16. A carbohydrate derivative according to any one of Claims 11–14, in which derivative at least a part of the carbaldehyde groups has been converted to a group with the formula $-CH(OR^3)-O-CH_2-COOR^2$ or $-CH(-O-CH_2-COOR^2)_2$, in which R^2 is hydrogen, a metal cation or an optionally substituted ammonium group, and R^3 is hydrogen or a direct bond to the oxygen atom of a dehydrogenated hydroxyl group of the carbohydrate.

17. A carbohydrate according to any one of the preceding claims, further containing carboxymethyl groups.

Abstract

A process for producing aldehydes or ketones is described, in which a primary or secondary alcohol, respectively, is oxidised using an oxidising agent and a catalytic amount of a nitroxyl compound, in the presence of a metal complex and/or an enzyme capable of oxidation. Further described are oxidised carbohydrates containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 50 monosaccharide units and per molecule.